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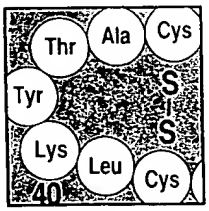
Experimental Hematology



Thrombopoietin accelerates platelet, red blood cell, and neutrophil recovery in myelosuppressed mice

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Abstract

The recent cloning of thrombopoietin (TPO) has allowed us to study its in vivo effects in normal and myelosuppressed mice. Normal Balb/c mice were treated with recombinant human TPO (hTPO) at doses ranging from 1 to 20 kU for 7 days, and complete blood counts (CBCs) and the number of megakaryocytes in the bone marrow were determined. Platelet counts were increased starting on day 5 after mice were treated with hTPO. Platelet counts reached a peak between days 8 and 11 and returned to baseline between days 16 and 20. hTPO treatment increased the number of megakaryocytes in the bone marrow starting on day 3. In normal mice, hTPO treatment did not affect red or white blood cell (RBC or WBC) counts. To test the effects of hTPO in myelosuppressed mice, Balb/c mice were irradiated with 350 cGy total-body irradiation and dosed with 1.2 mg carboplatin, resulting in severe and prolonged thrombocytopenia, anemia, and neutropenia. Treatment with 5–20 kU hTPO for 7 days accelerated the recovery of platelet, RBC, and neutrophil counts in myelosuppressed mice and also significantly improved their nadirs. In addition, bone marrow megakaryocyte numbers recovered 11 days earlier and reticulocyte counts recovered 10 days earlier in hTPO-treated myelosuppressed mice than in controls. These results indicate that TPO can improve hematopoietic recovery in myelosuppressed mice, affecting multiple cell lineages.

Key words: Thrombopoietin—Myelosuppression—Thrombocytopenia—Anemia—Megakaryocyte

Introduction

The major physiologic regulator of thrombocytopoiesis has long been believed to be a circulating hormone termed thrombopoietin [1–8]. Thrombopoietic activity is elevated in the plasma of thrombocytopenic animals. Recently, the protein responsible for this activity was cloned and shown to be a 70-kD glycoprotein [4–9].

TPO (also termed megakaryocyte growth and development factor [6], *mpl* ligand [4], or megapoietin [8]) is a protooncogene member of the hematopoietin receptor family [10,11]. TPO can stimulate megakaryocyte colony formation and increase the size, ploidy, and differentiation markers of megakaryocytic cells in vitro [5,12]. When administered to normal animals, TPO dramatically increases circulating platelet counts, with concomitant increases in marrow and splenic megakaryocyte number, size, and ploidy [12].

It has become increasingly evident that in mice, TPO treatment can affect not only the late states of megakaryocyte development but also progenitors of cells other than megakaryocytes. For example, recent studies have shown that TPO can influence the development of erythroid progenitor cells [13] in vitro, and that normal and myelosuppressed mice treated with TPO showed significantly higher myeloid progenitors of all lineages in the bone marrow and spleen than in vehicle-treated mice [14]. The purpose of this study was to evaluate the effects of TPO treatment on all lineages in normal and myelosuppressed mice.

Materials and methods

Mice

Seven- to 9-week-old female Balb/c mice (The Jackson Laboratory, Bar Harbor, ME) were maintained in AAALAS-certified animal care facilities with a 12-hour light-dark cycle. Acidified water and standard irradiated laboratory rodent chow (LM-485; Harlan Teklad, Madison, WI) were supplied ad libitum. Mice were housed in filter-top cages (five mice/cage) in a modified specific pathogen-free facility regularly monitored and free of common mouse pathogens (Murine Immuno-Comb; Charles River Laboratories, Wilmington, MA). Myelosuppressed mice were kept on circulating water heating pads. All procedures were reviewed and approved by the ZymoGenetics Animal Care and Use Committee.

Study design

The myelosuppressive regimen was adapted from that of Leonard et al. [15]. We used a more radiosensitive mouse strain (Balb/c) and reduced the total radiation dose. These modifications resulted in a similar degree of thrombocytopenia, neutropenia, and anemia but no mortality and minimal morbidity. On day 0, mice were exposed to 350 cGy total-body irradiation from a ^{137}Cs source (Gammacell 40 Irradiator; Nordion International, Kanata, Canada), immediately followed by an intraperitoneal injection of 1.2 mg carboplatin (Paraplatin; Bristol Myers Squibb, Cambridge, MA) reconstituted in 0.9% NaCl. Treatment with hTPO or vehicle started on day 1 and contin-

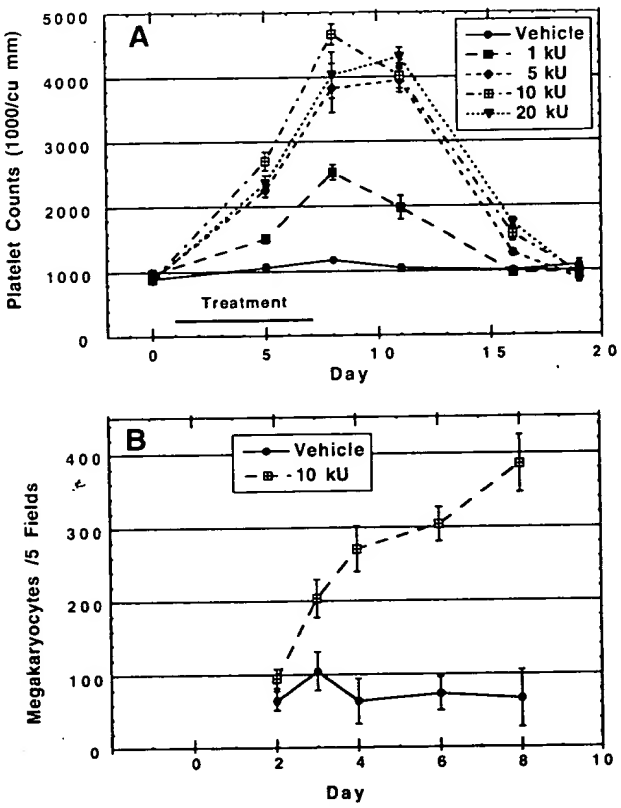

Fig. 1. Dose response of hTPO in normal mice. Mean circulating platelet counts are shown in **A**, mean bone marrow megakaryocyte counts in **B**. Mice received subcutaneous injections of hTPO daily for 7 days. Points represent mean ± SD (n=4/point).

ued through day 7. Doses of 1 kU/mouse were equivalent to 9 μg hTPO/kg body weight. hTPO was formulated in a vehicle of 0.05% polysorbate 80, 0.13 M NaCl, and 20 nM potassium phosphate (pH 6.0) and injected subcutaneously once daily. Two experiments were carried out, and the results were pooled to study the effect of hTPO treatment on blood parameters. Each treatment group consisted of 10 mice. To evaluate the effect of hTPO on bone marrow, and as a control for effects of multiple phlebotomies, additional mice were killed on specific days. These mice were bled only twice: 6–7 days before the start of the experiment and immediately before being killed.

Recombinant hTPO

hTPO was expressed in mammalian cells [16]. TPO from conditioned medium was purified by a combination of conventional techniques. From crude conditioned medium, TPO was captured and eluted from a mimetic green dye affinity column. The eluate from this column was then further purified by anion exchange chromatography and hydroxyapatite chromatography. Purified TPO migrated on SDS-PAGE gels at a molecular weight of approximately 70 kD and was essentially free of contaminating proteins. Protein concentrations were determined by amino acid analysis, and biological activity was assessed by the BAF-3 mitogenesis assay using BAF-3 cells engineered to express the human c-Mpl receptor. The quantity of TPO required to induce half maximal proliferation of mpl/BAF-3 cells was defined as 50 units. Endotoxin levels were ≤1.2 EU/dose.

Hematologic evaluation

Peripheral blood (60 μL) was collected from the retroorbital sinus under ether anesthesia into heparinized capillary tubes and immediately transferred to EDTA-coated microtainer tubes (Becton Dickinson, San Jose, CA). CBCs and differentials were performed on 50 μL blood with an Abbott Cell-Dyn 3500 hematology analyzer (Abbott Diagnostics, Santa Clara, CA) using mouse discriminator settings. Differential counts obtained from the hematology analyzer were verified to be within 10% of manual counts. Baseline values were collected for each mouse 6–7 days before irradiation/chemotherapy treatment and are shown on the graphs as day 0.

Reticulocyte counts

Reticulocytes were measured on a FACScan (Becton Dickinson). One microliter of blood was stained for 30 minutes at room temperature in the dark with Retic-Count (Becton Dickinson) according to the manufacturer's instructions. Unstained and stained cells were analyzed for each sample. Cells that were positive in the unstained sample were subtracted from the positive cells of the sample stained with thiazole orange. Reticulocyte indices were calculated for reporting the results.

Histopathology

Femurs were fixed in 10% neutral buffered formalin and decalcified in 20% sodium acetate and 10% formic acid. Paraplast X-TRA–embedded specimens were longitudinally sectioned at 2 μm and stained with hematoxylin and eosin for light microscopic examination. Each femur diaphysis was divided into five sections of equal size, and the number of megakaryocytes was counted under high power (40× objective) in one field/section.

Statistics

The effects of treatment on various parameters was analyzed using the nonparametric Kruskal-Wallis test followed by Dunn's multiple comparison test. A criterion of $p < 0.05$ was considered significant. Analysis was performed using Instat (Graphpad Software, San Diego, CA).

Results

Effect of TPO in normal mice

To determine the effect of different doses of hTPO on peripheral blood cell counts in normal mice, hTPO was injected subcutaneously daily for 7 days. Treatment resulted in a dose-dependent thrombocytosis (Fig. 1A). Platelet counts increased by day 5, reached a maximum between days 8 and 11, and returned to near normal values by day 20. A dose of 10 kU/day produced the maximal effect. To evaluate the effect of hTPO on the number and size of megakaryocytes in the femur, mice were treated daily with vehicle or 20 kU TPO and were killed on day 2, 3, 4, 6, or 8. The number of megakaryocytes increased significantly after 2 days of hTPO treatment and reached peak levels on day 8 (Fig. 1B). Figure 2 shows representative photomicrographs of bone marrow from hTPO-treated mice and vehicle controls, demonstrating the increase

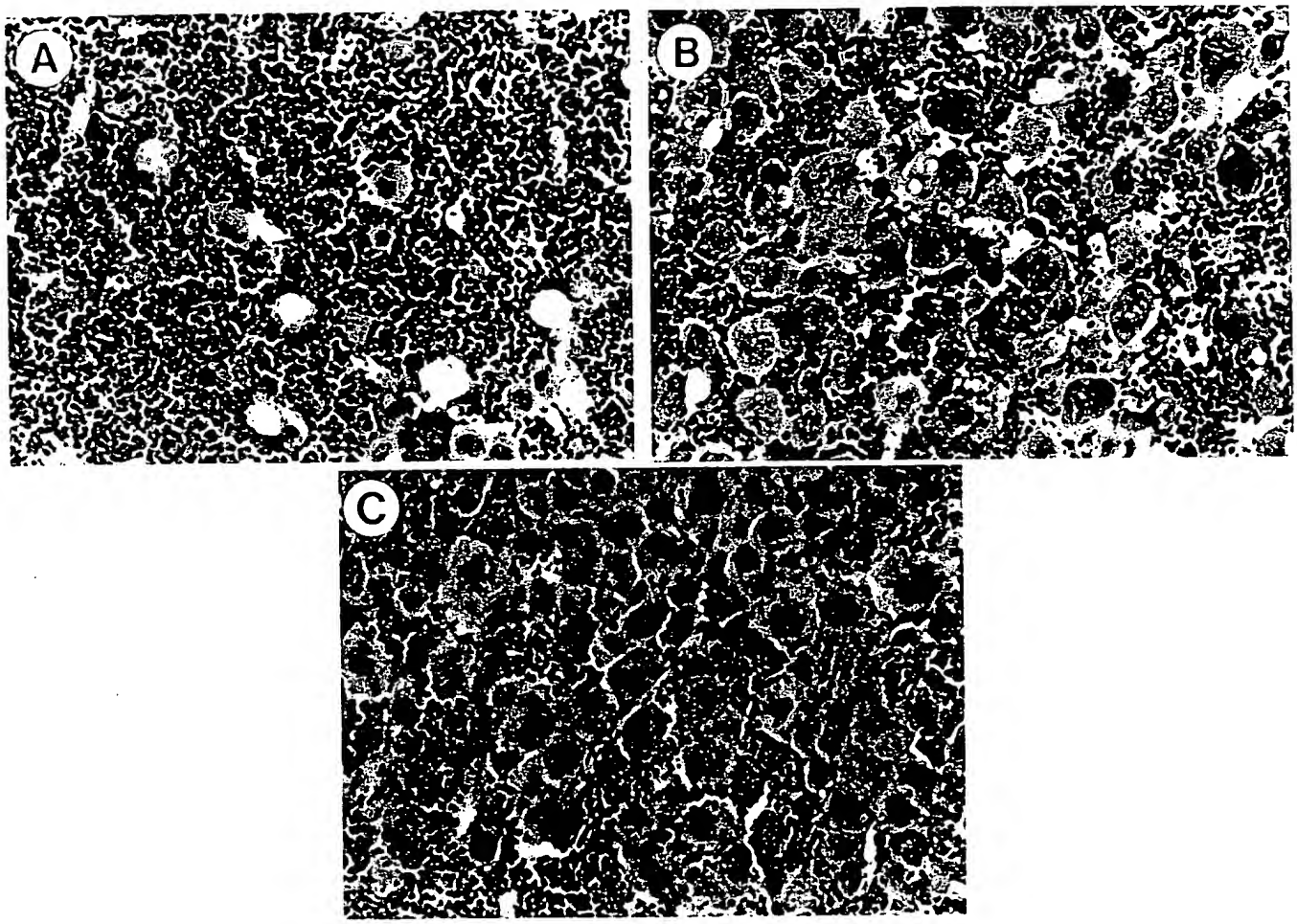

Fig. 2. Photomicrographs demonstrating the effect of hTPO on bone marrow megakaryocytes in situ in normal mice (H&E, 65×). Mice were treated with vehicle **(A)** or TPO (20 kU/mouse/day) for 3 days **(B)** or 6 days **(C)**. Mice treated with hTPO for 3 days were killed on day 4; all other mice were killed on day 8.

in megakaryocyte number in the hTPO-treated mice. None of the doses of hTPO had any effect on circulating RBC counts or absolute neutrophil count (ANC) in normal mice.

Effect of TPO in myelosuppressed mice

The combination of radiation and carboplatin resulted in reproducible, reversible thrombocytopenia, anemia, and neutropenia. Platelet counts were reduced to 7.6% of pretreatment values in vehicle controls by day 9, stayed at low levels for a week, and returned to normal values by day 20 (Fig. 3A). Vehicle controls also showed progressive anemia. RBC counts were lowest on day 20 and recovered to near-normal values on day 30 (Fig. 3B). Neutropenia was severe and lasted for at least 2 weeks (Fig. 3C).

Treatment with hTPO doses of 5 kU or more accelerated the recovery of circulating platelet counts in a dose-dependent manner (Fig. 3A). Mice treated with 5–20 kU showed significantly higher platelet counts at the nadir than vehicle controls (Table 1). In addition, the median time to 50% recovery of the baseline platelet count was 8–9 days faster in hTPO-treated mice (at doses of 5 kU or above) than vehicle-treated controls (Table 2). A rebound thrombocytosis was noted in the hTPO-treated groups as well as in the vehicle-treated mice.

Compared with controls, mice treated with hTPO doses of 5 kU or more had only a modest reduction in RBC counts. Treatment with hTPO (5–20 kU) resulted in significantly higher RBC counts at their nadir (Fig. 3B). In TPO-treated mice, RBC reached nadir levels on day 13; at higher TPO doses, RBC counts recovered back to baseline by day 16. This was accompanied by an earlier increase in peripheral blood reticulocytes in hTPO-treated mice. Recovery of the reticulocyte index started on day 6 compared with day 16 in the control group (Fig. 3C). Median recovery of reticulocytes to 100% of baseline was 18 days in the vehicle controls and 8–9 days in mice treated with 5–20 kU hTPO (Table 2).

Treatment with hTPO also modestly affected the recovery of neutrophils. At the nadir, the ANC was significantly higher in hTPO-treated mice (5 kU or more, Fig. 3D) compared with controls (Table 1). Additionally, recovery to 50% of baseline was significantly enhanced in the hTPO-treated mice (5 kU and more), and hTPO-treated mice recovered 7–12.5 days earlier compared with vehicle controls (Table 2).

Irradiation/carboplatin treatment did not affect the health of the mice; they showed normal grooming behavior and were

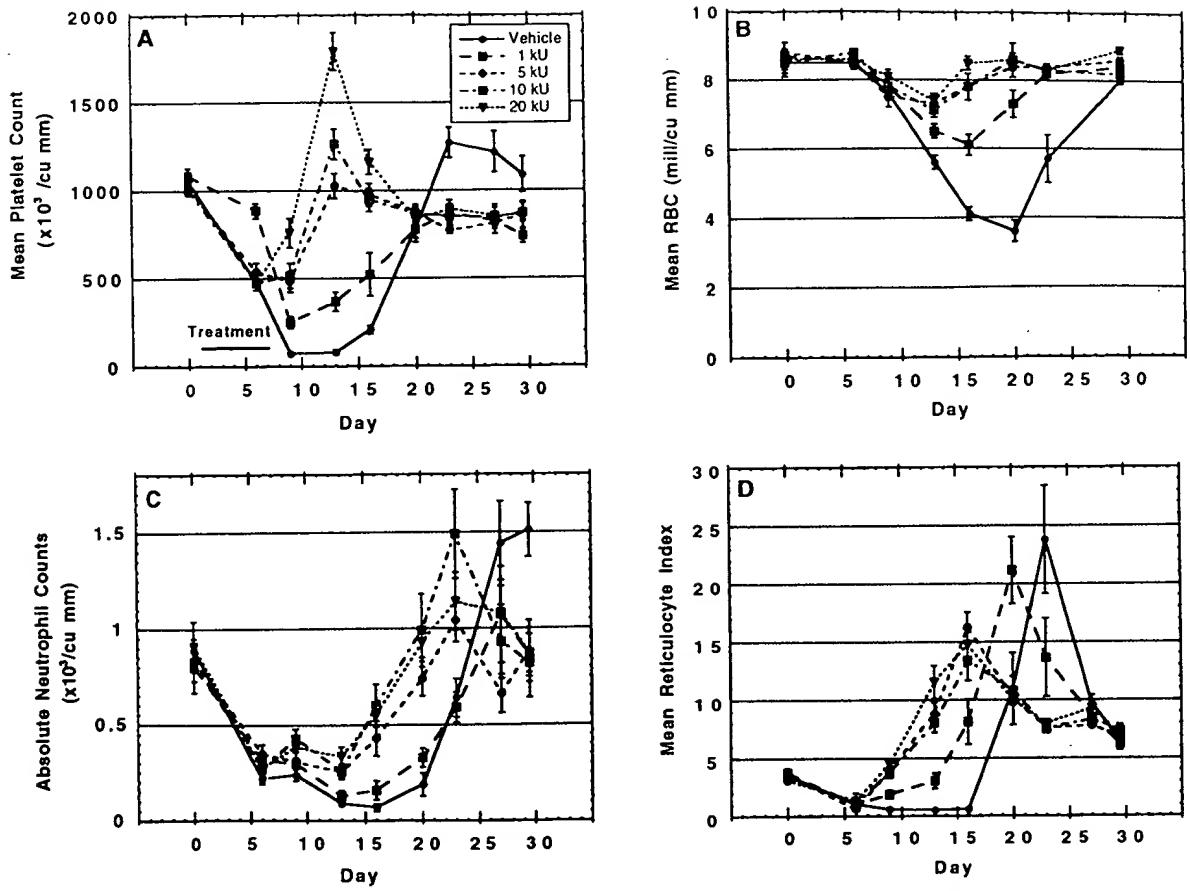

Fig. 3. Effect of 7-day hTPO treatment on platelet counts **(A)**, RBC **(B)**, ANC **(C)**, and reticulocytes **(D)** in myelosuppressed mice. Points represent mean ± SD (n=10/point).

Table 1. Nadir values of hematologic parameters in myelosuppressed mice

	Group[a]	No. mice	Median	Minimum	Maximum	*p* value
Platelet counts (1000/mm³)	Vehicle[b]	9	55	42	102	
	1 kU[b]	9	209	68	363	NS
	5 kU[b]	9	459	279	774	< 0.001
	10 kU[b]	10	474	209	618	< 0.001
	20 kU[b]	10	448	296	723	< 0.001
	Vehicle[c]	8	43	27	99	
	20 kU[c]	6	317	196	397	< 0.001
RBC (mL/mm³)	Vehicle[b]	9	3.4	2.5	4.5	
	1 kU[b]	9	6.4	4.9	7.3	NS
	5 kU[b]	9	7.4	6.3	7.6	< 0.001
	10 kU[b]	10	7.3	5.2	7.8	< 0.001
	20 kU[b]	10	7.2	6.6	8.4	< 0.001
	Vehicle[c]	5	6.0	4.83	6.9	
	20 kU[c]	6	7.3	70	8.2	< 0.001
ANC (1000/mm³)	Vehicle[b]	9	0.06	0.02	0.08	
	1 kU[b]	9	0.08	0.04	0.16	NS
	5 kU[b]	10	0.19	0.10	0.28	< 0.01
	10 kU[b]	10	0.20	0.12	0.36	< 0.001
	20 kU[b]	10	0.25	0.11	0.33	< 0.001
	Vehicle[c]	6	0.04	0.02	0.13	
	20 kU[c]	6	0.20	0.36	0.33	< 0.05

[a]Myelosuppressed mice were treated with the indicated doses of hTPO for 7 days. Blood cell counts and differentials were determined on the days shown in Figure 3. Baseline values (median): platelets 1.056×10^6/mm³; RBC count 8.59×10^6/mm³; ANC 840/mm³.

[b]Mice were bled at multiple time points, and the nadir of individual mice was used for statistical analysis (Kruskal-Wallis test followed by Dunn's multiple comparison test).

[c]Mice were bled only twice, precluding determination of nadirs for individual animals. The groups with the lowest median values (i.e., nadir) were used for statistical analysis (Mann-Whitney test).

Table 2. Recovery times for platelets, reticulocytes, and neutrophils in myelosuppressed mice

	Group[a]	No. mice	Median[b]	Minimum	Maximum	p value[c]
Platelet counts (days to 50% recovery)	Vehicle	9	18.5	19.5	20.5	
	1 kU	9	18.0	12.5	20.0	NS
	5 kU	9	9.5	6.0	12.5	< 0.01
	10 kU	10	7.5	6.0	11.0	< 0.001
	20 kU	10	9.5	6.0	13.0	< 0.01
Reticulocytes (days to 100% recovery)	Vehicle	9	18.0	15.5	25.0	
	1 kU	9	13.5	9.5	17.0	NS
	5 kU	8	9.0	7.5	10.5	< 0.01
	10 kU	8	9.0	6.5	13.0	< 0.001
	20 kU	9	8.0	7	9.0	< 0.001
ANC (days to 50% recovery)	Vehicle	9	21.5	20.5	26.5	
	1 kU	9	19.5	13.0	27.0	NS
	5 kU	9	14.5	7.0	18.5	< 0.05
	10 kU	9[d]	9.0	7.0	16.5	< 0.001
	20 kU	9[d]	13.5	6.5	23.5	< 0.01

[a]Myelosuppressed mice were treated with the indicated doses of hTPO for 7 days. Blood cell counts and differentials were determined on the days shown in Figure 3.
[b]The time to 50 or 100% recovery was extrapolated for each mouse from the values at the two time points surrounding either 50 or 100% of that mouse's baseline value and the time was then rounded to the nearest half day.
[c]TPO-treated groups were compared to the vehicle group for each parameter (Kruskal-Wallis test, followed by Dunn's multiple comparison test).
[d]One animal never decreased below 50% and was excluded from the analysis.

active. Mean body weights in myelosuppressed mice were slightly decreased (3–5%) on day 6 compared with baseline and returned to baseline by day 9. Mice that were necropsied at intermediate time points or at the end of the study showed no evidence of hemorrhage or other gross pathologic lesions.

Effect of TPO in mice bled only twice

To determine the effect of multiple phlebotomies on the results of hTPO treatment, separate groups of control and hTPO-treated mice were bled only twice, before the myelosuppressive treatment and before being killed. There was no difference in the recovery of platelet counts between control or hTPO-treated mice that were bled at multiple time points compared with mice bled only twice (data not shown). As expected, anemia was less profound in vehicle-treated mice bled only twice compared with vehicle mice bled multiple times. Twice-bled vehicle mice reached a nadir earlier than the animals with multiple phlebotomies (day 16 vs. day 20). In addition, RBC counts were higher in the twice-bled mice at the nadir (6.0 million/cu mm vs. 3.4 million/cu mm). Recovery and nadir of RBC counts, however, were virtually identical in hTPO-treated mice bled twice or multiple times (Table 1). Neutrophil counts of vehicle-treated mice bled only before being killed were very similar to mice bled multiple times. However, neutrophil counts recovered more slowly in hTPO-treated mice bled only before being killed compared with hTPO-treated mice bled multiple times (data not shown). Although the recovery was slower, there was still a significant difference between the neutrophil nadir of hTPO-treated mice bled before being killed and their vehicle controls (Table 1).

Effect of TPO on bone marrow recovery in myelosuppressed mice

To determine the effect of hTPO on bone marrow recovery, control and hTPO-treated myelosuppressed mice were killed on days 6, 9, 13, 16, and 20 (Fig. 4). On day 6, the megakaryocyte counts and the overall marrow cellularity were higher in the hTPO-treated mice compared with controls (Fig. 4). Three days later the cellularity had further increased in the hTPO-treated mice and began to increase in vehicle-treated animals. In contrast to vehicle controls, which had virtually no megakaryocytes in their bone marrow, the bone marrow of hTPO-treated mice had large numbers of megakaryocytes, and other erythroid or myeloid progenitor cells were also present. Even on day 13, mice treated with vehicle had very few megakaryocytes in their bone marrow. By this time, marrow megakaryocyte numbers had already peaked in the hTPO-treated mice and had begun to return to normal. Figure 5 illustrates the quantification of marrow megakaryocytes in these animals.

Discussion

TPO is a hematopoietic growth factor that acts in a relatively lineage-specific manner in normal animals. In myelosuppressed mice, however, TPO treatment accelerated the recovery not only of platelets but also of RBC and neutrophils. Several recent studies have shown that treatment of normal mice and monkeys with human or mouse TPO increases platelet counts in the peripheral blood [14,17,18]. In our study, doses of 1 kU/mouse (9 μg/kg) hTPO were effective in increasing platelet counts in mice more than two-fold. These results are similar to those reported by Ulich [18], who showed that doses of 5–10 μg/kg could increase platelet counts approximately two-fold. When species-specific TPO is used, even lower doses of TPO are required to obtain a platelet response. Doses of 2.5 μg/kg hTPO increased platelet counts in nonhuman primates [17] and 1 μg/kg mouse TPO is sufficient to increase platelet counts two-fold in mice (Grossmann, unpublished data). Differences in the doses of mouse and human TPO needed to increase platelet counts in mice are most likely related to decreased binding of hTPO to the mouse *mpl* receptor (data not shown).

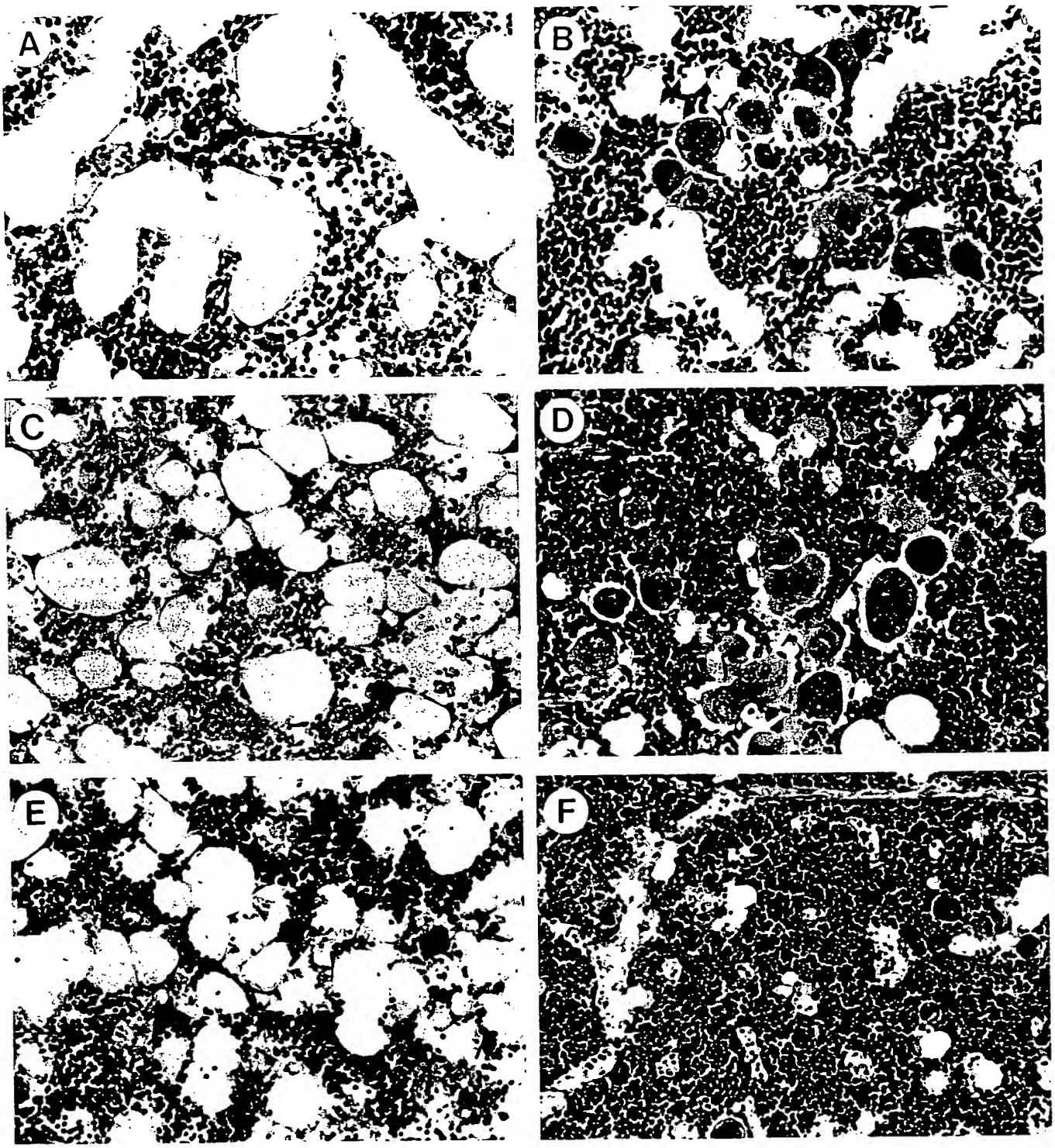

Fig. 4. Photomicrographs (H&E, 65×) showing the effect of hTPO treatment (20 kU/mouse/day) for 5 days (**A** and **B**) or 7 days (**C**–**F**) on bone marrow cellularity and the number of bone marrow megakaryocytes in situ in myelosuppressed mice. Mice were killed on day 6 (**A**: vehicle, **B**: TPO), day 9 (**C**: vehicle, **D**: TPO), and day 13 (**E**: vehicle, **F**: TPO).

Peak responses in platelet counts obtained after TPO treatment appear to depend on several parameters: dose, duration of dosing, receptor binding affinity, and possibly sex [19] (Sprugel, unpublished results). Mice treated for 7 days with human or mouse TPO reach a plateau at four to five times above baseline levels. A similar increase (six-fold) was reported by Farese et al. [17], who treated monkeys with different doses of hTPO for 10 days. In that study, 25 and 250 μg/kg also resulted in a similar peak response, indicating that high concentrations of TPO given over a relatively short period of time

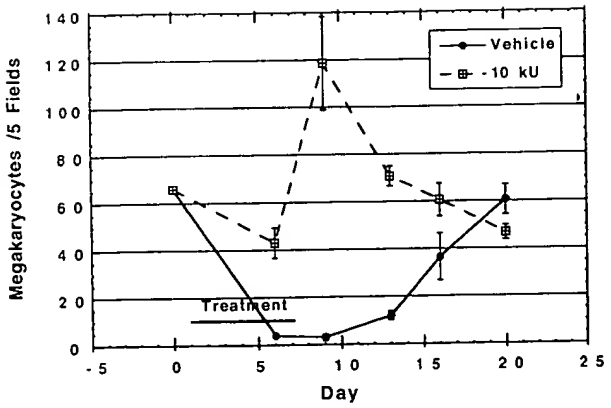

Fig. 5. Effect of hTPO treatment on the number of megakaryocytes in the femoral marrow space in myelosuppressed mice. Points represent mean ± SD (n=4–8/point). Mice received 7 daily subcutaneous injections of 20 kU hTPO.

do not further enhance peak responses. This inability to further increase platelet counts with short-term treatment may be related to *mpl* receptor downregulation or the ability of platelets to act as a sink for TPO. Even a single dose as high as 5 mg/kg did not increase platelet counts more than three-fold [18]. In contrast, treatment of monkeys for 28 days resulted in an increase in platelet counts up to 17-fold above normal (Sprugel, unpublished results), suggesting that peak responses can be increased if animals are treated for longer periods of time. This suggests that the control mechanisms that prevent extremely high platelet counts after short-term treatment can be overcome with longer treatment.

In previous studies, mTPO has been shown to enhance the proliferation of erythroid progenitor cells in the presence of IL-3 or c-Kit ligand (KL) and erythropoietin [13] and to expand the numbers of erythroid, myeloid, and megakaryocyte progenitors in normal mice [14]. In the TPO-treated normal mouse, however, it is likely that the cytokines required for the later stage of blood cell development are increased only for the megakaryocyte lineage (i.e., TPO). The full development of this expanded pool of progenitors to mature blood cells of all lineages may therefore be prevented. In contrast, in the myelosuppressed mouse, the cytokine environment might be considerably different.

To study the effect of TPO in myelosuppressed mice, we modified the model of Leonard et al. [15], which induces severe thrombocytopenia and anemia for a prolonged period of time using a carboplatin/irradiation regimen. As expected, TPO treatment accelerated the recovery of platelets in a dose-dependent fashion. Increases in platelet counts following TPO treatment have been previously shown in mice treated with carboplatin alone [18]; however, this model produced only a slight decrease in RBC (measured as hemoglobin), which was not significantly accelerated by TPO treatment. In contrast, the combination of carboplatin and irradiation can serve as a model of anemia as well as thrombocytopenia. Anemia is a later effect, most likely related to radiation injury. Although the mice did not hemorrhage internally in our study, part of the drop in RBC counts to low levels may be related to the multiple blood draws performed on each mouse. Indeed, mice bled only twice showed higher RBC counts then mice bled multiple times, suggesting that the severity of anemia was in part related to multiple blood draws. However, TPO treatment significantly reduced the anemia independent of the frequency of bleeding and induced reticulocytosis 1 week earlier than in control mice. In addition, TPO treatment of myelosuppressed mice increases the number of marrow BFU-E [14], establishing that at least part of the improved RBC levels is due to stimulation of erythropoiesis. Since RBC counts were virtually identical in the TPO-treated mice irrespective of the number of blood draws, the effect of TPO was not dependent on the degree of anemia. As in vitro studies indicate that TPO has direct and synergistic (with IL-3 or KL) effects on erythropoiesis [14], it is likely that at least part of the explanation for improved erythropoiesis during recovery from myelosuppression results from the interaction of TPO with high circulating levels of IL-3 and Epo induced by myelosuppression [20,21]. It is also possible that TPO-stimulated megakaryocytes produce cytokines affecting the development of other lineages. Megakaryocytes have been shown to secrete IL-3, GM-CSF, IL-6, and IL-1 [22]. Although it is not known whether TPO treatment increases the production of these cytokines by megakaryocytes in vivo, it is conceivable that the earlier recovery of megakaryocytes in the animals treated with TPO resulted in an increase of cytokines in the local bone marrow environment. High levels of IL-3 in myelosuppressed mice may also be able to stimulate the production of IL-3, GM-CSF, IL-6, and IL-1 by megakaryocytes [22].

TPO treatment affected both the neutrophil nadir and the time to recovery in the mice bled multiple times. This effect was greater in animals with multiple phlebotomies compared with animals bled twice. The cause for these differences is not clear but may be a secondary effect of other endogenous cytokines acting on precursor cells in combination with TPO and/or the effect of cytokine release induced by the myelosuppressive treatment regimen. In addition, although we have not seen clinical evidence of infection, local inflammation at the site of blood draw could induce inflammatory cytokines, which may act in concert with TPO to increase the number of neutrophils.

Recombinant TPO has been used in other models of myelosuppression. Hunt et al. [23], Shimosaka et al. [24], and Ulich et al. [18] have shown similar effects of TPO on platelet recovery. The effects on WBC and RBC counts have not been reported except in the model used here [13,14]. This suggests that the effect of TPO on nonmegakaryocytic lineages may be dependent on the severity of the myelosuppression. In the previous studies, myelosuppression models included treatment with several cycles of ACNU, single doses of carboplatin or 5-FU, or total-body irradiation [18,23,24]. None of these treatment regimens decreased platelet counts to levels as low as the combined radiation/carboplatin regimen and may not have induced severe anemia or neutropenia.

Treatment duration (7 days) in our study was shorter than in the other studies [18,23,24], ending 2 days before the platelet nadir. Despite this, platelet recovery was profoundly accelerated. The recovery of platelet counts without continuing TPO treatment may be explained by the time required for megakaryocyte maturation and platelet release. Furthermore, production of endogenous TPO may have aided in platelet

recovery. It has been shown in mouse and rabbit models that depleting platelets to below 30% of normal increased endogenous TPO levels [23,25]. In our model of myelosuppression, platelet counts dropped below 30% between days 6 and 9, probably inducing an increase in endogenous TPO levels. Hunt et al. [23] have shown in a similar myelosuppression model that endogenous TPO levels start to increase on day 8. Further stimulation of precursors may have occurred by endogenously produced TPO starting between days 6 and 9. In our hands, extending the treatment period with 10 kU hTPO to 14 days did not decrease the time to 50% recovery (data not shown); however, the rebound thrombocytosis was markedly increased (up to two-fold on day 16) compared with the shorter time of treatment (data not shown). This suggests that once endogenous TPO is produced at high levels, addition of exogenous TPO may be unnecessary to enhance recovery.

Several cytokines have been shown to increase platelet counts in myelosuppressed animals; however, these cytokines have generally been used at high doses. In a murine model similar to the one in our study, treatment of mice with IL-11 increased platelet counts. However, recovery was slower and IL-11 treatment was required for a longer time [15]. This IL-11 treatment also enhanced recovery of hematocrit and earlier recovery of reticulocytes [15]. In another model using cyclophosphamide-treated mice, administration of IL-11 also decreased the time to recovery of neutrophils and platelets [25]. Thus, like TPO, IL-11 produces effects in myelosuppressed animals that are not lineage-specific.

Another cytokine that has been shown to alter platelet recovery in myelosuppressed animals is IL-6. High doses of IL-6 [27] decreased the duration of irradiation- or 5-FU-induced thrombocytopenia in mice; however, nadir counts were unchanged. Similarly, irradiated dogs [28] and primates myelosuppressed with chemotherapy [29] had earlier platelet count recovery when treated with IL-6. But despite these favorable effects on hematologic recovery, most clinical studies using IL-6 have been associated with toxicity, including acute phase response and worsening anemia [30,31]. The effects of TPO observed in our study are both quantitatively and qualitatively greater than those reported for IL-6, and were associated with no apparent hematologic or other toxicity.

The results from this study demonstrate that administration of hTPO to myelosuppressed mice reduces not only the severity and duration of thrombocytopenia but also the severity of anemia and neutropenia. Clinical trials will establish whether comparable effects can be obtained in patients with thrombocytopenia, anemia, and/or neutropenia associated with myelosuppression and bone marrow transplantation.

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